Unveiling the sensory connections between the bladder and the brain that involve the periaqueductal gray matter

Valorization

In this thesis, the role of periaqueductal gray (PAG) matter in mediating the afferent bladder signals using well-established expertise, is examined. This project already needed interdisciplinary knowledge utilization and the results will certainly open new avenues where knowledge is collected, interpreted and utilized in an interdisciplinary framework in the future. From a scientific and clinical point of view, there is a foreseeable possibility of identifying novel mechanisms of brain function that would facilitate the accurate targeting of the micturition control sites in the brain, and management of the symptoms in patients with micturition abnormalities. Specifically, understanding the essence and extent of PAG involvement in local and remote neural elements that regulate bladder sensation and micturition behavior will clarify the main components driving the therapeutic benefits of clinical interventions, and the mechanisms that facilitate and that work at cross-purposes in patients. The knowledge utilization aspects of this thesis can be outlined as follows:

Opportunities for Drug Discovery

In chapters 4 and 5 we investigated the involvement of various neurotransmitter systems in ventrolateral column of the PAG regarding the reception of bladder sensory signals. These experiments revealed the glutamatergic neurotransmission to be the main cellular mechanism mediating these inputs. Glutamatergic neurotransmission has excitatory influence over its target regions, and has been under extensive pharmacological investigation. Here, we found out a substantial target for therapeutic applications (Table 1).

Note that most of the drugs mentioned in table 1 are active either in epileptic or neurodegenerative disorders. Particularly the latter may affect the PAG as well. Besides this, many vacant drug mechanisms exist with potential for synthesis of novel agents. For example drugs which act over synthesis or storage of the glutamate, or those that may act on reuptake or degradation of the released drug into the synapse. Each potential agent may act by facilitating or inhibiting either phase. On the other hand, receptor level mechanisms may be directed towards investigating specific glutamate receptor subtypes, or designing drugs which have agonist, partial agonist or reverse agonist mechanisms of action. Further investigation will have to focus on finding out the exact location of specific glutamate receptor subtypes in specific PAG columns.

These drugs will certainly be active not only on the urological patient, but on all individuals who suffer from dysfunctions in the spectrum of PAG functions including nociception, behavioural and autonomic systems. As detailed above, the impact of this pathway is abundant both because the PAG has a broad spectrum of functions, and there

are a lot of drugs active in this system. Thus, any pharmacological intervention in this system in the PAG requires detailed understanding of the associated micro-circuitry and need a more or less systems physiological approach. Additionally, one may investigate specific side effects or potential benefits that each of the already discovered drugs (Table 1) have on urodynamic function. Particularly for clinical investigators, that would be a great opportunity to expand on.

Table 1. So far clinically tested drugs or agents active on the glutamatergic system can be classified as follows (some drugs have multiple mechanisms of action):

- Glutamate release inhibitors :
  - Levetriacetam
  - Riluzole
  - phenobarbital
- Glutamate receptor antagonists
  - NMDA antagonists:
    - NO
    - Xe
    - PCP
    - Ketamine
    - Alcohol
    - acamprosate
    - Methadone
    - Amantadine
    - memantine
    - Riluzole
    - Dizocilipine [MK 801]
    - Felbamate
  - AMPA antagonists:
    - phenobarbital
    - Topiramate
    - lamotrigine
    - Philanthotoxin [produced by Wasps]

Potential targets for surgical intervention

Furthermore, there is a possibility of modulating PAG function by deep brain stimulation (DBS). In contrast to peripheral neuromodulation, central neuromodulation has not been performed for urological disorders. This approach particularly has the advantage of treating the central etiologies when they exist, for example in extrapyramidal disease with micturition symptoms, rather than symptomatic treatment. Moreover, there are promising future techniques for neuromodulation of micro circuits such as DREADD (designer receptors exclusively activated by designer drugs), or magnetic stimulation using nano-particles, which may ultimately turn central neuromodulation into a non-

invasive technique. Additionally, new DBS paradigms have the advantage of being on demand, with a patient controlled interactive system. This decreases the adverse autonomic effects, as well as enabling the patient to increase the amount of stimulation in certain circumstances. Defining and programming for different types of stimulation, for example a change of stimulation frequency or application of burst algorithms, would also allow to specifically restore a particular function. The latter is a promising and an only sporadically explored field in urological applications for neurostimulation.

Diagnostic applications

In chapter 2 we elaborated on some common diseases affecting the PAG and compromising the bladder sensation and micturition. We also showed that many of these pathologies can be detected by modern imaging techniques, especially specific MRI techniques such as fMRI. This ultimately lead us to the conception that, the PAG must be included in future diagnostic and therapeutic patient management algorithms. This would make clinicians aware of the possible involvement of this structure in pathophysiology of disease, and guide them to more appropriate decisions. More precise evaluation of patient condition may be achieved by Magnetic Resonance Spectroscopy (MRS), to find out the chemical composition of a particular PAG segment. This would aid in the diagnosis of many diseases. For example, it may aid in defining the state of the involvement of glutamatergic PAG cells in multiple system atrophy, and correlating it with the severity of symptoms. With the upcoming high resolution MRI machines, we can gain more insight about the specific columns of the PAG.

Novel preclinical techniques

In addition to the above mentioned clinical derivatives that may result from this thesis, we introduced two techniques to be used by researchers in the associated fields.

In chapter 3, we introduced a method for peripheral neuromodulation in rodents, which would be more comfortable for the animal and for the experimentalist. This method may be used by allied research groups which have an interest in chronic peripheral stimulation, recording or monitoring in live rats, like those working in urological, gastrointestinal and spinal research. This technique in general, engages an access point over the rat skull and redirects the connections from the subcutaneous space.

In chapter 6, we described a slight technical modification for observation of the pontamine sky blue dot. The pontmaine sky blue dye is used for localization of a glass micropipette electrode. The very small injection area adjacent to the tip of the electrode, accompanied by light scattering in bright field microscopy, makes the visualization difficult. On the other hand, we exploited the fluorescence of pontmaine to observe minute injection quantities in the dark field fluorescence microscope. This increases the contrast to a great deal and makes the visualization much easier. To aid the background

clarification we used Hoechst staining. This technique may be readily used by other electrophysiologists using glass micropipettes as recording electrodes.

Our translational neuromodulation group at MUMC will facilitate direct utilization of the scientific outcomes in clinical practice. This group, comprised of integrated collaboration between the departments of neuroscience, neurology, neurosurgery, anesthesiology, psychiatry, urology, colorectal surgery and ENT, provides a unique team to conduct translational urodynamic/neuromodulation studies. Close collaboration and communication between basic scientists and clinicians in our team will lead to a successful implementation of preclinical findings in clinical practice.

Our novel insights will be shared with patient organizations, health care professionals and scientific societies. From an academic perspective, our results have been/will be published in peer-reviewed international journals and will be presented at (inter)national scientific meetings